Pathogens and Toxins in Foods: Challenges and Interventions
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Chapter 6

Listeria monocytogenes

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TYPE OF ILLNESS AND CHARACTERISTICS OF THE ORGANISM

In 1924 in Cambridge, England, what is now known as *Listeria monocytogenes* was first documented by E. G. D. Murray and colleagues (1926) as the causative agent of a septic illness, peripheral monocytosis, in laboratory rabbits. A few years later Nyfeldt (1929) reported the first case of illness in humans that was attributed to this bacterium. *L. monocytogenes* is a gram-positive, non-spore-forming rod that exhibits tumbling end-over-end motility at 22°C. Biochemically, it is catalase positive and oxidase negative, and it hydrolyzes esculin and exhibits slight β-hemolysis on blood agar (Ryser and Marth, 1999). There are currently 13 serotypes (1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, and 7) (Khelef et al., 2006). The optimum growth temperature is 30 to 37°C, but it can grow at −0.4 and 45°C and can survive freezing for prolonged periods (Rowan and Anderson, 1998). The optimum pH range for growth is pH 6 to 8, but growth has been observed between pH 4.4 and 9.6 (Pearson and Marth, 1989; Ryser and Marth, 1999). In general, the pH that will support growth of *L. monocytogenes* increases as the temperature decreases. *L. monocytogenes* can also survive and/or grow at water activity (a_w) levels that are usually too low for other bacteria to survive, that being a_w values of ≤0.911 (Nolan et al., 1992). The ability to survive and/or grow over a wide range of temperatures, pH, and a_w values is of great concern to the food industry, because this provides an opportunity for the pathogen to survive in a variety of niches at various points within food processing operations and ultimately contaminate product contact surfaces and finished products (Tompkin, 2002).

LISTERIOSIS

There is still much that is not known about how this disease is manifested in the human body (McLauchlin, 1996). Because the pathogen is opportunistic and is not necessarily adapted to humans, it probably has multiple routes of infection and types of symptoms, depending on how it enters the body; however, the principal route of infection for food-borne listeriosis begins with the ingestion of contaminated food. Although healthy adults and children can also suffer from listeriosis, young, old, pregnant, and immune-compromised individuals ("YOPIs") are particularly susceptible (CDC, 2000). Of the estimated 2,500 food-borne cases per year in the United States, of which about 500 are fatal, about one-third involve pregnant women (Mead et al., 1999). Symptoms range from an asymptomatic infection to flu-like symptoms, such as fever and muscle aches in healthy individuals, to septicemia and meningitis in immune-compromised patients, intrauterine infections in pregnant women, and severe systemic infections in the unborn or neonates. Until recently, it was thought that pregnant women were more susceptible because their immune system was changed and/or compromised during pregnancy. New information indicates that once the pathogen has been ingested and disseminated to the maternal organs, it is able to migrate to the placenta, wherein it is protected from the maternal immune system (Bakardjiev et al., 2006). Between 1976 and 2002, there were 27 outbreaks of food-borne listeriosis reported worldwide, with about 2,900 cases and about 260 deaths (mortality rate of ca. 9.0%) (McLauchlin et al., 2004). Between 1998 and 2002, although listeriosis represented only 0.7% of bacterial food-borne illnesses over this 5-year

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period, it was responsible for 54% of all deaths (CDC, 2006). The use of genomic and proteomic tools has generated appreciable insight into the genes and proteins that make *L. monocytogenes* virulent. Such progress has also been accelerated by the availability and comparison of the whole genomes of select serotype 4b and 1/2a strains (Nelson et al., 2004; Glaser et al., 2001). More detailed information on molecular characterization of the determinants involved in invasion of host cells, intracellular motility, and cell-to-cell spread is beyond the scope of this chapter but can be found elsewhere (Kathariou, 2002; Paoli et al., 2005). Further expansion of our knowledge of the mechanism(s) of pathogenesis at the molecular level will ultimately result in more direct and more effective interventions to better manage the occurrence, persistence, and numbers of this pathogen in our food supply.

SOURCES AND INCIDENCE OF *L. MONOCYTOGENES* IN THE ENVIRONMENT AND FOODS

L. monocytogenes is widespread in nature, being associated with plants, soil, water, sewage, feed, and animals raised as food (CFSAN, 2003). It has also been recovered from stool samples of an estimated 1 to 10% of healthy humans (Farber and Peterkin, 1991). The pathogen has also been recovered from a variety of raw and ready-to-eat (RTE) foods (Meng and Doyle, 1997). The majority of cases linked to foods are associated with refrigerated, RTE foods that were consumed without reheating. Refrigerated, RTE foods provide an ideal environment for *L. monocytogenes*, primarily because the pathogen can grow at low temperatures, whereas competitors cannot.

The bacterium is found in numerous foods with a prevalence ranging from 13% in raw meats to 3% in dairy products, 11% in fresh vegetables, and 3% in seafoods. When found in RTE foods, levels are typically low. For example, Gombas et al. (2003) reported a prevalence of 1.82% (577 of 31,705 samples) and levels of <0.3 most probable numbers (MPN)/g to 1.5×10^5 CFU/g in six categories of RTE foods. Likewise, Wallace et al. (2003) reported a prevalence of 1.6% (532 of 32,800 samples) among 12 brands of commercial frankfurters and levels of 1.0 to 5.0 $\log_{10}$ CFU per package/pound. As summarized by Farber and Peterkin (1991), when found in various meat and dairy products, the pathogen is usually present at levels of ca. 1.0 to 3.0 $\log_{10}$ CFU/g but sometimes can be found at levels as high as 7.0 $\log_{10}$ CFU/g. A Joint Agency risk assessment prioritizing the relative risk of listeriosis among 20 food categories identified deli meats, undercooked chicken, and Latin-style cheese as foods of higher risk (CFSAN, 2003). Efforts to fill research voids needed to conduct risk assessments are aided by the ability to predict the fate of the pathogen on various foods using the tools of predictive microbiology. In this regard, ComBase, the Pathogen Modeling Program (PMP), and the Predictive Microbiology Informational Portal are worth mentioning. ComBase is a relational database that contains over 40,000 data sets/records of the growth, survival, and/or inactivation of *L. monocytogenes* and other food-borne pathogens under diverse environments relevant to food processing operations. The PMP is a stand-alone online application used by ca. 30% of the food industry for hazard analysis of critical control point validation. It is accessed by ca. 5,000 online users in some 35 countries worldwide each year to predict the fate of pathogens and spoilage microbes in a variety of foods. The PMP contains some 40 models, including 23 models for growth, 3 for heat inactivation, 4 for survival, 4 for cooling, and 6 for irradiation. The Predictive Microbiology Informational Portal is a comprehensive website that presently is comprised of predictive models, research data, relevant regulatory policies, and guidelines pertaining to *L. monocytogenes* and RTE meat and poultry products (http://portal.arserrc.gov).

INTRINSIC AND EXTRINSIC FACTORS THAT AFFECT SURVIVAL AND GROWTH IN FOOD PRODUCTS AND CONTRIBUTE TO OUTBREAKS

The psychrotrophic nature of *L. monocytogenes* is of particular concern, given that refrigeration is one of the most common interventions used to ensure safety and extend shelf-life (Gandhi and Chikindas, 2007) and primarily because RTE foods, such as Latin-style cheese and deli meats, may be consumed without any reheating and/or further preparation. *L. monocytogenes* growth/survival at low temperatures requires maintenance of membrane fluidity for appropriate enzymatic activity and transport of solutes across the membrane, as well as for structure stabilization of macromolecules, such as ribosomes, and/or the uptake or synthesis of compatible cryoprotectant solutes, such as glycine betaine and carnitine (Chattopadhyay, 2006; Gandhi and Chikindas, 2007). *L. monocytogenes* may exhibit a thermotolerance response when exposed to temperatures above its optimum growth temperature, given that these temperatures may trigger physiological responses that induce synthesis of polypeptides, namely, heat shock proteins (Rowan and Anderson, 1998). Many factors affect thermotolerance of *L. monocytogenes* in foods,

such as the proximate composition of the food; differences in the strains used or their physiological state; presence of deleterious chemicals in the growth media; environmental stresses, such as osmotic and acidic shocks; history/adaptation of the cells prior to inoculation; and the microbiological media and/or incubation conditions used to recover injured cells (Doyle et al., 2001).

Acidic stress may also increase the ability of the pathogen to survive in foods and/or may facilitate expression of its virulence genes (Ryser and Marth, 1999). The minimum pH requirement reported for *L. monocytogenes* growth in foods is pH 4.4, when at near-optimum temperatures. However, its growth at low pH is dependent on the medium or food composition, the strain and its physiological state, and the preincubation temperature (Phan-Thanh et al., 2000). For example, Parish and Higgins (1989) reported that *L. monocytogenes* survived for 21 and 5 days at 4 and 30°C, respectively, in orange serum samples adjusted to pH 3.6 with HCl. Its ability to adapt to acidic environments is of particular concern because the pathogen encounters these environments in low-pH foods, such as fermented meat and dairy products; in the acid conditions within the gastrointestinal tract; and in the phagosomes of macrophages (O'Driscoll et al., 1996; Cotter and Hill, 2003). The adaptation of *L. monocytogenes* to low-pH environments is the result of the activation of stress adaptation mechanisms, such as induction of proteins, pH homeostasis, the glutamate decarboxylase system, sigma factor (σ^B), and a two-component regulatory system, comprised of *lis*R and *lis*K (Shen et al., 2006; Gandhi and Chikindas, 2007). Tolerance of acidic environments can be induced in *L. monocytogenes* by exposure to sublethal pH. O'Driscoll et al. (1996) reported that, regardless of the acid used to adjust the pH, acid-adapted (pH 5.5) cells of *L. monocytogenes* strain LO28 showed greater tolerance toward lethal pH (pH 3.5) compared to those that were not acid adapted (pH 7.0). As another example, Greenacre et al. (2003) reported that cells of *L. monocytogenes* strain EGD-e also developed an acid tolerance response at 20°C when cells were acid adapted (pH 5.0 to 5.5) with either acetic acid or lactic acid prior to exposure to a lethal pH (pH 3.0).

The lowering of a_w in foods is another strategy widely used for controlling food-borne pathogens. Ingham et al. (2006) evaluated the survival of *L. monocytogenes* on vacuum-packaged beef jerky and related products stored at 21°C for up 28 days. The authors reported that jerky products with an a_w of $\leq$0.87 did not support growth of *L. monocytogenes,* but the organism survived in jerky at a_w values $\leq$0.47. The a_w of foods can be altered by addition of osmolitic compounds, by removal of water using physical methods, and/or by binding of water to a range of macromolecular components (Stecchini et al., 2004). The stress response of *L. monocytogenes* to a low-a_w environment is induced, at least in part, by accumulation of solutes, such as glycine betaine and carnitine (Bayles and Wilkinson, 2000).

Studies have shown that glycine betaine and carnitine are the most effective solutes for osmotic adaptation of *L. monocytogenes* (Bayles and Wilkinson, 2000; Angelidis and Smith, 2003). The ability of *L. monocytogenes* to accumulate these intracellular solutes allows it to grow at NaCl concentrations of $\leq$12% (Razavilar and Genigeorgis, 1998), thus challenging control of this pathogen in salted RTE foods, such as soft cheese and deli meats. The intracellular solutes that are synthesized or that are taken up from the food or natural environment compensate for the external osmotic strength without affecting the macromolecular structure of the cell (Duché et al., 2002; Smith, 1996). As one example, Smith (1996) evaluated the adaptation of osmotically stressed cells of *L. monocytogenes* on processed meat surfaces. Their results showed that*L. monocytogenes* accumulated higher levels of glycine betaine and carnitine when inoculated onto the surfaces of bologna, frankfurters, wieners, ham, and bratwurst at refrigerated temperatures than when inoculated in liquid media supplemented with either of the osmolytes. According to Garner et al. (2006), exposure of *L. monocytogenes* to environmental conditions present in RTE meats, such as the addition of NaCl and organic acid salts, may enhance its pathogenicity and consequently the association of listeriosis with these foods.

FOOD PROCESSING OPERATIONS THAT INFLUENCE THE NUMBERS, SPREAD, OR CHARACTERISTICS

Control of *L. monocytogenes* is one of the most difficult challenges faced by manufacturers and handlers of RTE meat, poultry, seafood, and dairy products. Overcoming this challenge requires the flawless execution of numerous food safety-related systems and operations, including proper sourcing and storage of ingredients, segregation of raw product areas from cooked/pasteurized product areas, heat treatment or other listericidal steps, sanitary design of equipment and facilities, sanitation, environmental monitoring, and cold chain management. Other important control considerations, such as the intrinsic and extrinsic factors of the food and specific *L. monocytogenes* control interventions, are addressed elsewhere in this chapter.

While most other microbial food-borne pathogens can be controlled by a well-validated cooking or pasteurization step, *L. monocytogenes* thrives in the cool, damp environment typical of the postcook or postpasteurization environments of RTE manufacturing facilities, retail slicing areas, and even home refrigerators. Thus, protection of cooked or pasteurized RTE products from recontamination by *L. monocytogenes* and from exposure to conditions conducive to *L. monocytogenes* growth is key to protecting public health.

Sourcing and Storage of Ingredients

A consideration of all inputs to the process or product is a key step in controlling *L. monocytogenes*. Ingredients in which *L. monocytogenes* is capable of multiplication should be stored at temperatures and for times that will not allow for an increase in numbers of the pathogen that might subsequently overwhelm the final kill step. Ingredients added after cooking, such as sauces, dry spice rubs, and heat-sensitive nutrient supplements, are even more critical. If there is a regulatory or public health-determined "zero tolerance" for *L. monocytogenes* in the final product, ingredients added postcook must also be free of any viable *L. monocytogenes*. Processing aids, packaging materials, and brine chill solutions, as well as water and air, should also be carefully considered if they will be contacting the post-*L. monocytogenes*-kill-step product.

Segregation of Raw and Treated Areas

Because *L. monocytogenes* is ubiquitous in the environment and is often present on raw ingredients, it is important to minimize the presence and levels of *L. monocytogenes* in the posttreatment environment, especially when the product is directly exposed to that environment. Complete exclusion of *L. monocytogenes* is not attainable, but limiting the occurrence of *L. monocytogenes* in the posttreatment environment to the minimum level possible will greatly protect the treated/finished product (Tompkin, 2002). The RTE industry has used numerous practices to attain this goal. For example, segregation of employees that work in raw versus treated areas can best be attained by having dedicated employees for each area. Many facilities even provide separate employee welfare areas (locker rooms, cafeterias, and restrooms) to avoid cross-contamination. For many operations, employing two complete sets of workers is impractical. Carefully planned procedures then become critical for limiting *L. monocytogenes* cross-contamination when employees transition from raw to treated areas. One of the most important considerations is provision of footwear and outer clothing that are worn only in the high-care areas and that are adequately cleaned and sanitized on a routine basis. A well-designed hand wash station incorporating such things as touchless water, soap, paper towel, and final hand sanitizer dispenser controls and with comfortable water temperature and adequate sink space (so employees are not rushed) is just one of the recommended requisites. Separate sets of tools, utensils, and equipment for production, maintenance, and sanitation should be maintained for the pretreatment and posttreatment areas. Manufacturers should also consider how ingredients, packaging materials, sanitation equipment and supplies, garbage/inedible containers, pallets, quality assurance supplies, reworked product, fork lifts/hand trucks, lubricants, and other processing aids will enter the treated product area. Air pressure should be highest in the most critical high-care area, becoming more negative in the raw areas. Air balances should be conducted to ensure that these pressures are maintained during all phases of operation, especially including the sanitation shift, when the generation of microaerosols could spread *L. monocytogenes*. Air balances should be repeated whenever new equipment is added or whenever the facility is modified in ways that could impact the air balance. Facility air chilling systems should be designed so that *L. monocytogenes* is not able to multiply within the system or be disseminated in the air stream. Modern systems that treat the air supply with heat, HEPA filtration, and/or UV radiation are available. These systems must also be designed to quickly reduce the humidity generated during sanitation and maintain low humidity levels during operation.

Kill Step

The capacity to kill *L. monocytogenes* during the cooking or pasteurization step has been determined for many products and processes (CDC, 1988; Juneja and Eblen, 1999). This kill capacity is not unlimited, so worst-case estimations of the levels potentially present before the kill step should be conducted to assess the adequacy of this control point. Baseline studies conducted by the USDA/FSIS indicated the highest levels of *L. monocytogenes* detected in several types of raw meat and poultry (Table 1). Unfortunately, public data do not exist for many meat and poultry ingredients, nor do they exist for most other ingredients, such as spice blends. If any ingredients added prior to cooking are suspected of having occasional high levels of *L. monocytogenes*, quantitative testing should be conducted to determine maximum levels. A critical limit for the level of kill necessary to produce a safe product needs to be determined based

Table 1. Levels of *L. monocytogenes* found in USDA baseline sampling[a]

Type of animal product (sample type)	No. sampled	Highest level (MPN/cm^2)
Market hogs (carcass swab)	2,112	46
Broiler chickens (carcass rinse)	1,297	51
Cows and bulls (carcass swab)	2,112	43
Young turkeys (carcass rinse)	1,221	0.3

[a]Source: http://www.fsis.usda.gov/Science/Baseline_Data/index.asp. Accessed 15 April 2008. MPN, most probable numbers.

on a worst-case estimation of the load of *L. monocytogenes* that could potentially be in the product before the kill step. These data can be generated by conducting quantitative testing of the uncooked product or ingredients, or adapted from existing published data (Table 1). It is generally accepted that the heating times and temperatures necessary to eliminate *Salmonella* in cooked meat and poultry products are also adequate to eliminate *L. monocytogenes*. Traditional milk pasteurization times/temperatures are also considered generally adequate to eliminate the typically low levels of *L. monocytogenes* that may be present in raw milk. Once validated, however, the kill step must undergo routine verifications to ensure its continuing efficacy.

Sanitary Design of Equipment and Facilities

The most typical cause of the contamination of RTE product with *L. monocytogenes* is from a growth niche that exists in or near product contact surfaces on equipment in the exposed posttreatment product area. A growth niche is an area that contains the essential ingredients of food, water, and time within the *L. monocytogenes* growth temperature range. The food source is typically residue from the product being manufactured, although it can sometimes come from other sources, such as dust from a cardboard box. Water can be supplied from numerous sources, including juices from the product, residual water left from the cleaning/sanitation process, discharged chilling water from packaging equipment, leaking steam and water valves, condensation, and even poorly managed antimicrobial foot baths or floor foaming systems. The time necessary for *L. monocytogenes* growth depends on temperature and other extrinsic conditions. The most problematic areas of equipment that can become growth niches for *L. monocytogenes* are the areas that do not receive physical disruption (scrubbing, turbulent flow of clean-in-place systems, and high-pressure spray) and/or do not receive adequate exposure to cleaning and sanitizing chemicals. Examples of growth niches are hollow equipment elements that are not hermetically sealed (support structures, conveyor rollers, motors, etc.), gaskets, pumps, rubber floor mats, bearings, drive chains, cracks, bolt/screw/rivet penetrations, electrical panels, equipment controls, compressed air lines, and cooling coils. Anytime two solid pieces of material are combined, if moisture can infiltrate the junction, the potential exists for an "*L. monocytogenes* sandwich." The most common harborage sites for *L. monocytogenes* are typically the floor and drain systems. The most effective way to limit the levels of *L. monocytogenes* in the overall facility environment is to strive for clean, sealed, unbroken floors that remain dry during food production shifts. Excellent information on best practices for the sanitary design of equipment and facilities has been compiled by the American Meat Institute and is available at www.meatami.org (accessed 15 April 2008).

Sanitation

Sanitation, especially in critical high-care areas, is one of the most important *L. monocytogenes* control points. Cleaning is the critical step that removes food residues and allows the chemical sanitizer to function optimally. A four-step cleaning process is recommended. The first step is dry cleaning, removing as much product residue as possible by scraping, sweeping, and shoveling. Care should be taken to pick up and dispose of this residue and not rinse it down the drainage system, where clogs and backups can occur, leading to a high-risk *L. monocytogenes* situation. The second step is the rough rinse with hot water (approximately 140°F). This temperature is important, because if it is too cool, then fats and oils will not be melted and removed, and if too hot, then protein residues can be baked onto the equipment. The third step is applying the appropriate detergent. This detergent should be matched to the application by a cleaning chemical expert, as it needs to match the cleaning needs (clean in place, foaming, scrubbing, etc.), water softness, residues to be removed, etc. Detergent is commonly applied as a foam solution. It is important that the foam not be allowed to dry on the equipment, as it will not rinse properly if allowed to dry. The final cleaning step is the final rinse, where it is critical that all remaining food and detergent residue be completely rinsed from the equipment. When the equipment and room are clean, the appropriate sanitizer can be applied. The most important

consideration for sanitizer application is coverage. Flood sanitizing (low-pressure and high-volume) application of the sanitizer is most effective. Other methods (fogging and garden sprayer) may not give 100% coverage and should be avoided, except in special circumstances. Many facilities double and even triple sanitize to ensure for the most complete kill of *L. monocytogenes* and other microorganisms. Often, a fast-acting oxidizing sanitizer, such as a mixed peracid, is used first and is followed by a quaternary ammonium solution that is slower acting but has residual killing activity. Most sanitizers should be applied as a mixture with cool water. If regulations allow, the final sanitizer should be applied at a level that can be left on equipment without a final rinse.

For very difficult-to-sanitize equipment, heat can be effectively used to kill *L. monocytogenes*. Equipment can be covered with a tarp and steamed, placed in an oven, or immersed in hot water. The sanitizing method should be carefully evaluated for employee safety and degradation of heat-sensitive machine components. Clean-out-of-place tanks can be very useful for cleaning and heat sanitizing smaller parts. Heat sanitizing processes should be validated for the ability to kill *L. monocytogenes* in the central, most heat-protected areas of the equipment. When using wet heat, the times and temperatures necessary to kill *L. monocytogenes* in food products will also kill *L. monocytogenes* in or on equipment. Dry heat has much less killing capacity, so higher temperatures and longer application times are typically required.

Environmental Monitoring

Areas capable of harboring *L. monocytogenes* are not always visually apparent. Environmental monitoring gives manufacturers the ability to find and eliminate *L. monocytogenes* harborage sites and growth niches. The two biggest keys to success are aggressive searching and diligent corrective action. One recommendation for aggressive searching is to reward employees for finding positive environmental samples. Others include using the fastest, most sensitive methods possible; sampling large areas; and sampling areas that are the most difficult to reach and clean. Using a broader indicator group, such as *Listeria* spp. or *Listeria*-like organisms, enhances the ability to find growth niches, in contrast to looking specifically for *L. monocytogenes*. The RTE meat and poultry industry has found the greatest success in finding *L. monocytogenes* growth niches by doing routine monitoring several hours after the start of processing operations or at the end of production but before sanitation. Sampling devices should be optimized to pick up the target group from the equipment so that it can be transferred into the detection system. Microcellulose sponges, single-ply tissues, and gauze all work well and pick up the target group more efficiently than cotton-tipped swabs or direct-contact plates (Vorst et al., 2004). A neutralizing buffer should be used to moisten the sampling device. Samples should be kept refrigerated and be processed in a timely manner to prevent overgrowth by competitors or die-off of the target group.

Cold Chain Management

Protection from *L. monocytogenes* growth conditions does not end when the product leaves the manufacturing facility. If the product is temperature abused at any step of manufacture, storage, distribution, retail, or home use, the potential for *L. monocytogenes* to grow to infectious numbers increases; thus, the key to minimizing listeriosis is to prevent *L. monocytogenes* multiplication (Chen et al., 2003). Many manufacturers occasionally send temperature monitoring devices with the product during transit and storage and then review the monitoring information to ensure that the cold chain is routinely being maintained. In addition, retailers and display case manufacturers have made improvements in maintaining cold temperatures during retail display. A joint risk assessment conducted by the FDA and the USDA indicated that if all home refrigerators could be maintained at or below the recommended 41°F, cases of listeriosis could be cut by >98%, highlighting the importance of the cold chain in minimizing this disease (CFSAN, 2003).

RECENT ADVANCES IN BIOLOGICAL, CHEMICAL, AND PHYSICAL INTERVENTIONS TO GUARD AGAINST THE PATHOGEN

L. monocytogenes is arguably the food-borne pathogen that has consistently garnered the most regulatory attention over the past 25 years. The severity and mortality of the disease, the nature and number of the most susceptible members of our society, and the frequency and magnitude of product recalls have resulted in the "zero tolerance" policy in effect at present in the United States, that being an allowable limit of ≤1 cell/25 g food (0.04 cell/g) (Shank et al., 1996). However, as pointed out by Buchanan and colleagues (1997), it is noteworthy that the relative frequency of listeriosis in countries that practice zero tolerance, such as the United States, is about the same as that in countries that do not, such as Germany (about 4 or 5 cases per million inhabitants). Despite advances that reduced by about 40% both the prevalence of the pathogen in certain

foods and the occurrence of listeriosis (CDC, 2006), additional guidelines are in place for producers of red meat and poultry products (Anonymous, 2003). This ruling provides manufacturers with three options for the plant/product which ultimately determine the frequency of regulatory testing: use of both a postprocess lethality step and an antimicrobial to control outgrowth (lowest testing frequency) (alternative 1); use of either a postprocessing lethality step or an antimicrobial to control outgrowth (moderate testing frequency) (alternative 2); or use of appropriate sanitation alone (most testing) (alternative 3). Due to this "incentivized" offering by USDA/FSIS, efforts have intensified to develop and implement biological, chemical, and/or physical interventions to control the pathogen both pre- and postprocessing. In addition, USDA/FSIS also provides a venue for companies to implement antimicrobial approaches via plant trials to provide efficacy data for USDA acceptance that can be listed on the USDA's New Technologies web page, along with a description of the antimicrobial process (http://www.fsis.usda.gov/Regulations_&_Policies/New_Technologies/index.asp).

BIOLOGICAL INTERVENTIONS

Bacteriophage

Several companies have proposed utilizing bacteriophage as a biological anti-listerial intervention for food and other applications (Intralytics, Inc., Baltimore, MD; OmniLytics, Salt Lake City, UT; EBI Food Safety, Wageningen, The Netherlands). One virulent lytic bacteriophage, phage P100, displayed a host range against 95% of the 250 *Listeria* isolates tested (Carlton et al., 2005). When the bacteriophage was applied to soft cheese, Carlton et al. (2005) observed a 2.0- to 3.0-$\log_{10}$ decrease in viable counts of inoculated *L. monocytogenes* (1.5×10^8 PFU/ml phage titer), which improved to a 3.5-$\log_{10}$ reduction with higher phage titers (3×10^9 PFU/ml). This has been developed into a product, Listex-P100 (EBI), that has been granted GRAS (generally recognized as safe) status by the FDA on a petition submitted by Intralytics and that has been granted approval as a food additive for use on RTE meat and poultry products (FDA, 2006).

Bacteriocins

Bacteriocins are inhibitory peptides and proteins produced by bacteria. Those produced by lactic acid bacteria have long been proposed as food "biopreservatives" because of the historical use of these organisms in food fermentations, their generalized GRAS status for use in foods, and their inhibitory activity against various food-borne pathogens (Muriana, 1996). Use of purified bacteriocins would constitute a direct food additive and require FDA approval. The only bacteriocin that has been granted such approval is nisin, which is allowed for use in low-moisture/low-salt pasteurized processed cheese. Other bacteriocins have been used in this regard by employing bacteriocin-producing lactic acid bacteria for production of cultured milk or whey preparations that are used directly as ingredients. In efforts to expand the application of nisin as an antimicrobial, researchers have been examining its use in a variety of different food applications, for which it has not yet been approved. Using nisin-coated plastic films for vacuum-packaged cold-smoked salmon, Neetoo et al. (2008) have shown a 3.9-$\log_{10}$-CFU/cm^2 reduction of *L. monocytogenes* relative to growth in controls at the highest level of nisin used (2,000 IU/cm^2). Concern has also surfaced for the resistance of *L. monocytogenes* to nisin and other bacteriocins that may occur during repetitive selective pressure in food applications.

PHYSICAL INTERVENTIONS

High-Pressure Processing

High-pressure processing (HPP) represents a promising nonthermal process for the preservation of sensitive products, such as sliced deli meats, that are prone to quality changes using typical thermal processes. Pressures of 100 to 600 MPa are being used to control microbial growth at low or moderate temperatures, without affecting organoleptic properties. The level of inactivation by HPP depends on the type of microorganism, pressure, treatment time, temperature, pH, water activity, and the composition of foodstuffs (Hugas et al., 2002). Cellular functions sensitive to pressure include modification of membrane permeability, fatty acid composition, cell and membrane morphology, protein denaturation, and inhibition of enzyme activity; however, a change in membrane structure is believed to be the main cause of inactivation (Lado and Yousef, 2002). Several disadvantages of HPP are batch-wise processing, the cost of scale-up to handle commercial-sized quantities of product, and the fact that not all organisms are equally affected.

Irradiation

Irradiation, also known as cold pasteurization, is an effective control measure in maintaining the quality of raw, cooked, and minimally processed meat products (Molins et al., 2001). The FDA/WHO Codex

Alimentarious Commission considers irradiation a safe technology for controlling *L. monocytogenes* in raw and uncooked meat. Among the different forms of irradiation, UV, gamma radiation, and electron beam (generated by electricity) are considered to be bactericidal. Because of their poor penetration power, UV rays are restricted to the treatment of food or equipment surfaces and eradication of airborne contaminants and, thus, can be of some practical use in reducing *L. monocytogenes* in food production and storage areas. In 2001, 40 countries permitted the use of irradiation in different types of foods, including 12 countries that allow irradiation for control of pathogens in poultry, 8 countries that permit its use in meat, and 13 that allow its use for fish and seafood (Molins et al., 2001). Zhu et al. (2005) demonstrated a 2.0- to 5.0-$\log_{10}$ reduction of *L. monocytogenes* on hams at 1.0 to 2.5 kGy, respectively, while examining electron beam irradiation of RTE products in the presence of various acidulants. Although flavor changes of raw meats induced by irradiation may be masked during cooking, flavor and quality challenges still exist for its application to RTE meat products.

Thermal Surface Pasteurization

Listeria contamination on RTE products often represents postprocess surface contamination, for which treatment of the entire mass of product may not be necessary. Submersed-water, postpackage pasteurization has been used commercially as a postprocess lethality step for surface pasteurization of RTE deli products capable of providing a 2.0- to 4.0-$\log_{10}$ decrease after 2 to 10 min of exposure at 91 to 96°C (Muriana et al., 2002). Radiant heat prepackage surface pasteurization has also achieved similar lethalities of *L. monocytogenes* (2.0- to 2.8-$\log_{10}$ reduction) in less time (60 to 75 s), with reduced purge loss or need for special pasteurization bags and reduced chilling requirements (Gande and Muriana, 2003). Prepackage pasteurization requires the product to be packaged immediately upon exit from the oven to eliminate the possibility of recontamination; however, a combination of the two processes eliminates this aspect while providing a 3.0- to 4.0-$\log_{10}$ reduction of *L. monocytogenes* (Muriana et al., 2004). Either method alone provides an alternative 2 process or in combination with chemical antimicrobials could provide for an alternative 1 process.

Other Physical Methods

As reviewed elsewhere in detail (NACMCF, 2006), additional methods that could be used as microbial interventions may also include microwave processing, ohmic heating, pulsed electric fields, nonthermal plasma, oscillating magnetic fields, ultrasound, and even filtration.

CHEMICAL INTERVENTIONS

Organic Acids

Many organic acids have GRAS status as food ingredients, and studies have shown their effectiveness under various conditions and in different foodstuffs. Such chemicals are more effective when used at a pH below the pK_a of the acid. Under such conditions, more of the undissociated form of an organic acid can enter cells and dissociate to generate the toxic anion, thus inhibiting the organism. The problem with many foods in which this form of inhibition takes place is that direct application of the acidulant may create a temporary acidified film around the product, which may be absorbed and neutralized with time by the food itself. The combination of the sodium/potassium salts of lactate and diacetate has become the standard ingredient for the processed meat industry ever since the USDA/FSIS increased the allowable level of lactate to 4.8% and of diacetate to 0.25% of the total formulation (Anonymous, 2000). Additional research demonstrated that this combination of acidulants had appreciable inhibitory activity for suppressing the growth of *L. monocytogenes* in formulated/processed RTE meats (Barmpalia et al., 2004; Bedie et al., 2001; Stekelenburg, 2003).

Oxidizing Solutions

Ozone is a strong oxidizing agent approved with GRAS status for the sanitization of bottled water in 1982. More recently, it was approved as an antimicrobial agent on food, including meat and poultry (Anonymous, 2001). As a general oxidant, it attacks chemical/organic constituents of the cell wall/membrane of bacteria and other microbes, including *L. monocytogenes*. The use of ozone is limited by its short half-life, which requires that it be used quickly upon generation. When foods are treated directly, its effectiveness is also limited by the degree of organic material, which reduces the oxidizing capacity of the solution, and therefore, it shows more consistency in application on surfaces with minimal organic background, such as fruits, vegetables, and environmental surface sanitation. Wade et al. (2003) found a greater efficacy of the reduction of *L. monocytogenes* (1.48 $\log_{10}$ CFU/g) on alfalfa sprouts and seeds that were continuously sparged for 5 to 20 min with ozonated water than that on those treated with water alone.

Smoke-Derived Flavorings and Extracts

Traditional smoking of commercial food products has been replaced largely by "liquid smoke" treatment because of the quick and easy application; however, liquid smoke is used mostly for flavor attributes and does not adequately provide the preservation characteristics of traditional smoked products. Liquid smoke is known to possess a variety of phenolic compounds that have demonstrated inhibitory properties toward pathogens and spoilage organisms. Faith et al. (1992) demonstrated the ability of liquid smoke to inactivate *L. monocytogenes* in hot dog exudates. In further testing with 11 different phenols, isoeugenol, a major phenolic component of liquid smoke, was the only phenol shown to appreciably inhibit *L. monocytogenes* (Faith et al., 1992). Other components derived from liquid smoke, including carbonyl compounds, have also demonstrated effective antilisterial properties on RTE meats and have suppressed growth of *L. monocytogenes* when inoculated at low levels or provided as much as a 5.3-$\log_{10}$ reduction when combined with heat pasteurization (Gedela et al., 2007a, 2007b).

Lauric Arginate

Current practices, such as the use of postprocess thermal treatments and/or application of biological and food-grade chemicals, have met with varying levels of success at reducing the prevalence of this pathogen. Regarding the latter, most studies evaluated the delivery of food-grade chemicals as an ingredient and/or a bath, dip, or spray for the finished product, and/or that were applied into or onto the packaging material. Instead, the sprayed lethality in container (SLIC) method was developed to introduce an antimicrobial solution into the packaging container just prior to when the finished meat or poultry product is placed within the package and then rely on the vacuum-packaging step to evenly distribute the antimicrobial purge, such that total coverage of both product and package is achieved (Luchansky et al., 2005). Depending on the product, this system was effective at delivering about a 2.0- to 5.0-$\log_{10}$ reduction of *L. monocytogenes* within 24 hours at refrigeration temperatures. Relative to other interventions that use food-grade chemicals, food treated via SLIC tastes better because there is less impact on flavor. Moreover, production costs are appreciably reduced to about $0.002 per pound, resulting in cost savings of $0.5 to $2 million per year for a small- or medium-sized frankfurter processing plant. Additionally, using this system would result in significantly shorter processing times (2 or 3 s), and the compact SLIC system fits directly on existing production lines at a nominal, one-time cost of $5,000 to $10,000 for the equipment.

DISCRIMINATIVE DETECTION METHODS FOR CONFIRMATION AND TRACE-BACK OF CONTAMINATED PRODUCTS

Subtyping offers an approach for investigating the relatedness of isolates and identifying and tracing the sources of epidemics (Lyytikäinen et al., 2000; de Valk et al., 2000; Sim et al., 2002). Subtyping has also been of great value in identifying sources of contamination in food processing plants (Berrang et al., 2000; Rørvik et al., 2003). As summarized elsewhere (Ryser and Marth, 1999), there are several practical (cost and ease-of-use) and scientific (typeability, reproducibility, and discriminatory power) considerations for selecting a suitable method for subtyping. The more often a particular method is practiced, the easier and less costly it becomes. Regarding scientific criteria, all strains should be unambiguously typeable by the method(s) used, the same results should be obtained each time by all persons using a standardized method, and strains that are indistinguishable should be shown as such, whereas strains that are nonidentical should be shown as different. Regardless, the results obtained with a given typing method must also be epidemiologically relevant (Tenover et al., 1997). The literature is replete with various methods for typing and/or tracking listeriae (Sauders et al., 2006). Although all methods provide useful information, some methods are better than others, relative to typeability, reproducibility, and/or discriminatory capability (Arbeit, 1995). For example, despite a general usefulness in studies of food-borne illness, it is often difficult to differentiate isolates of *L. monocytogenes* using only phenotypic methods: between 20 to 40% of strains are non-phagetypeable, the same or similar O antigens are found in several species/strains, serotype 4b strains are responsible for all major outbreaks and about 50% of sporadic cases, and the majority of the strains involved in food-borne outbreaks comprise two distinct genomic divisions that correlate with flagellar antigens (Rocourt, 1994). Thus, research on the implementation and optimization of typing strategies, used alone or in combination, has intensified to establish unequivocal relationships among strains for molecular subtyping studies.

It became quite apparent by the late 1980s that the overwhelming number of food-borne listeriosis cases were caused by only three serotypes, namely, serotypes 1/2a, 1/2b, and 4b. Thus, serotyping has limited value for epidemiologic applications. Another

approach that was used to type *L. monocytogenes* was multilocus enzyme electrophoresis; isolates were grouped into one of two types (based on their enzyme locus profiles), I (4b, 1/2b, and 3b) and II (1/2a and 1/2c), which also coincided with groupings based on flagellar antigens a and c and on b (Piffaretti et al., 1989). Interestingly, isolates from sporadic cases of listeriosis and animal isolates could not be grouped into distinctive subsets based on multilocus enzyme electrophoresis types. In the mid-1990s the techniques of pulsed-field gel electrophoresis and ribotyping confirmed that *L. monocytogenes* grouped into these same two distinct groups (Brosh et al., 1994). Over the past decade, nucleic acid sequence-based typing schemes have flourished, given the advancements in PCR amplification and sequencing technologies. Automated DNA sequencers with laser fluorescence detection using a single gel lane can provide upwards of 500 bp of DNA sequence for as little as US$10.00 (core facility charge). One common method is multilocus sequence typing, whereby specific genetic loci are identified, amplified, sequenced, placed together as artificially contiguous DNA sequences, and analyzed in comparison to similar loci from other strains by multiple sequence alignment analyses (Maiden et al., 1998). One of the main advantages of multilocus sequence typing over DNA fragment-based typing schemes is the elimination of the ambiguity in fragment migration, especially in databases containing gels of DNA fragments produced by scientists in different labs. Automated DNA sequencing is more accurate than ever before, and DNA sequences are readily analyzed by portable computer software for simultaneous comparisons of various clones.

There are numerous examples already published describing the use of molecular methods to subtype and source *L. monocytogenes* on farms, in plants, and/or with foods. Due to space constraints, we will illustrate the approach using a study that we recently completed (Brito et al., 2008). Based on the association of *L. monocytogenes* with raw and pasteurized milk, dairy farms, cheese processing plants, and Latin-style cheese and, in turn, on the potential threat of listeriosis, we used pulsed-field gel electrophoresis to identify possible sources of contamination in a dairy processing plant producing Minas frescal cheese (MFC). Samples from 9 of 10 MFC brands from retail sites located in Juiz de Fora, Minas Gerais, Brazil, tested negative for *L. monocytogenes;* however, 6 of 10 from "brand F" of MFC tested positive. Thus, the farm/plant that produced brand F MFC was sampled; several sites within the processing plant and MFC samples tested positive. All 344 isolates recovered from retail MFC, plant F MFC, and plant F environmental samples were serotype 1/2a and displayed the same AscI or ApaI fingerprints. These results helped establish that the storage coolers served as the contamination source of the MFC. Following renovation, samples from sites that previously tested positive for the pathogen were collected from the processing environment and MFC on multiple visits; all tested negative for *L. monocytogenes*. Our results validated that systematic culturing and analyses of products and processing facilities can identify areas that harbor *L. monocytogenes*. In addition, we also demonstrated that it is possible, with appropriate interventions, to eliminate harborage points within a food processing facility.

CONCLUDING REMARKS

Although in theory and from a policy perspective one can talk about eliminating *L. monocytogenes* from our food supply, given its ubiquity, persistence, and pathogenicity, in practice it may be more a question of better managing efforts to identify harborage points and applying interventions to lessen the prevalence and levels of this pathogen and, in turn, the threat to public health. To this end, we must be ever vigilant in following appropriate good manufacturing practices, good agricultural practices, standard operating protocols, and hazard analysis and critical control point programs, so as to minimize the load and occurrence of the pathogen, and concomitantly continue efforts to develop and implement effective interventions to ensure that an infectious dose of *L. monocytogenes* will not reach the consumer's plate. We must also be pragmatic with regard to when, where, and how much of our resources should be directed toward developing policies and hiring inspectors versus directed toward filling research voids. A wealth of information collected from the numerous studies conducted thus far confirms that some foods present a greater risk for listeriosis due to their ability to support survival/growth of the pathogen (e.g., $\geq$pH 4.4, $\geq a_w$ 0.92, $\geq$4°C, absence of inhibitory substances, and/or combinations thereof) and that some people (i.e., "YOPIs") are at greater risk due to their age and/or health status. As such, it may be time to revisit the zero tolerance policy and consider allowing minimal levels of *L. monocytogenes* (e.g., $\leq$100 cells/g) in lower risk foods that do not support its growth. This would allow food safety professionals to focus available resources on foods that present a greater threat to human health and to ostensibly reduce the occurrence and severity of listeriosis worldwide.

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